APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1993, p. 2730–2733 Vol. 59, No. 8
0099-2240/93/082730-04$02.00/0


# Rapid Mini-Prep Isolation of High-Quality Plasmid DNA from *Lactococcus* and *Lactobacillus* spp.†

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Received 10 December 1992/Accepted 29 May 1993

**A simple, rapid plasmid mini-prep procedure for lactococci and lactobacilli which gives high yields and can be performed on overnight broth cultures is presented. Large plasmids were isolated from both lactococci and lactobacilli, including a 70-kb plasmid from *Lactobacillus acidophilus* C7. The purity of the resulting plasmid DNA makes it suitable for subsequent molecular manipulations. The convenience of the technique makes this rapid mini-prep procedure suitable for routine plasmid isolation from lactic acid bacteria.**

Physical evidence for plasmid DNA in bacteria was first presented in the early 1960s from the results of buoyant-density centrifugation in the presence of intercalating dyes and electron microscope techniques (reviewed in reference 5). The discovery of plasmid DNA in the lactic acid bacteria is generally attributed to Cords et al. (3) and has since been correlated with a number of commercially associated phenotypes in lactic acid bacteria, including lactose metabolism, proteinase activity, citrate fermentation, bacteriocin production, drug resistance, sugar transport and metabolism, and the phage resistance mechanisms of restriction/modification, adsorption resistance, and abortive infection (reviewed in references 4, 8, and 10).

Procedures for the detection and analysis of plasmids in bacteria were greatly simplified by the development of agarose gel electrophoresis methods for plasmid DNA (11), applied to the lactic acid bacteria by Klaenhammer et al. (9). An efficient lysis procedure was concurrently developed for strong-walled gram-positive bacteria, involving growth of cells in the presence of 20 mM DL-threonine to weaken the cell wall, facilitating lysozyme degradation of the wall and subsequent sodium dodecyl sulfate (SDS)-mediated lysis of the cell membrane (2).

A procedure for rapid purification of lactococcal plasmid DNA suitable for restriction endonuclease analysis was subsequently provided by Anderson and McKay (1). This method was based on alkaline-mediated denaturation of chromosomal DNA and extraction of protein with salt-saturated phenol and chloroform-isoamyl alcohol. It is very effective and is still used in many laboratories, but it is quite different from *Escherichia coli* plasmid mini-prep procedures, requires specialized materials (e.g., salt-saturated phenol), and does not fully eliminate chromosomal DNA. In this communication, we present a simpler, faster method which is analogous to *E. coli* mini-prep procedures and thus can be easily accommodated by most laboratories. Moreover, our procedure can be performed on overnight (or older) cultures and will yield sufficient quantities of pure plasmid DNA (high or low copy number) for restriction analysis and cloning experiments.

The following bacterial strains were used in the course of this study: *Lactococcus lactis* strains NCK545, NCK546 (NCK culture collection, this lab), NCK203, NCK211 (6), and LM0230, containing the high-copy-number vector plasmid pIL253 or the low-copy-number plasmid pIL252 (14); *Lactobacillus plantarum* NCK129 and *Lactobacillus delbrueckii* subsp. *bulgaricus* 10A (NCK culture collection), *Lactobacillus acidophilus* C7 (7), and *Lactobacillus johnsonii* VPI11088 (NCK88) (12). Lactococcal strains were cultured at 30°C in M17 broth (Difco Laboratories, Detroit, Mich.) containing 0.5% glucose. For LM0230, NCK211, and NCK546, erythromycin (2 μg/ml) was incorporated to maintain selection for recombinant plasmids. *Lactobacillus* strains were cultured in MRS broth (Difco Laboratories) at 37°C. All agarose gels were electrophoresed horizontally in 0.5× Tris-borate buffer (13).

The details of the plasmid mini-prep procedure used in this study are outlined in Fig. 1. This procedure was devised by modifying steps from *E. coli* mini-prep procedures to meet the specific needs of lactococcal plasmid isolation. The initial lysozyme step, which is generally not necessary for gram-negative bacteria, is imperative here. The high concentration of lysozyme used was found to be very efficient at protoplasting, with no adverse effects. It should be noted that while this treatment was sufficient to protoplast lactococcal strains, some *Lactobacillus* spp. may be better treated with mutanolysin, as described by Klaenhammer (7). To test this hypothesis, the protoplasting solution in step 1 was supplemented with 300 U of mutanolysin; this enabled complete lysis of most of the *Lactobacillus* spp. used. However, this modification was found to be detrimental to detection of the larger *Lactobacillus* plasmids and is therefore not generally used for plasmids of >25 kb.

Mixing of the DNA solution immediately after the addition of SDS enabled a more thorough distribution of the detergent and facilitated more efficient lysis of the cell population. The degree and severity of mixing (in all steps) were dictated by the sizes of the plasmids present; for example, vortexing for a few seconds was used for small plasmids (<25 kb), but to prevent shearing of larger plasmids, gentle mixing was accomplished by several tube inversions, sufficient to achieve mixing. The presence of ethidium bromide facilitated protein extraction by phenol-chloroform, presumably by intercalating the DNA and enabling displacement of tightly bound proteins (15). The presence of ethidium bromide also promoted a very tight and coherent interface during the phenol-chloroform extraction, which greatly sim-

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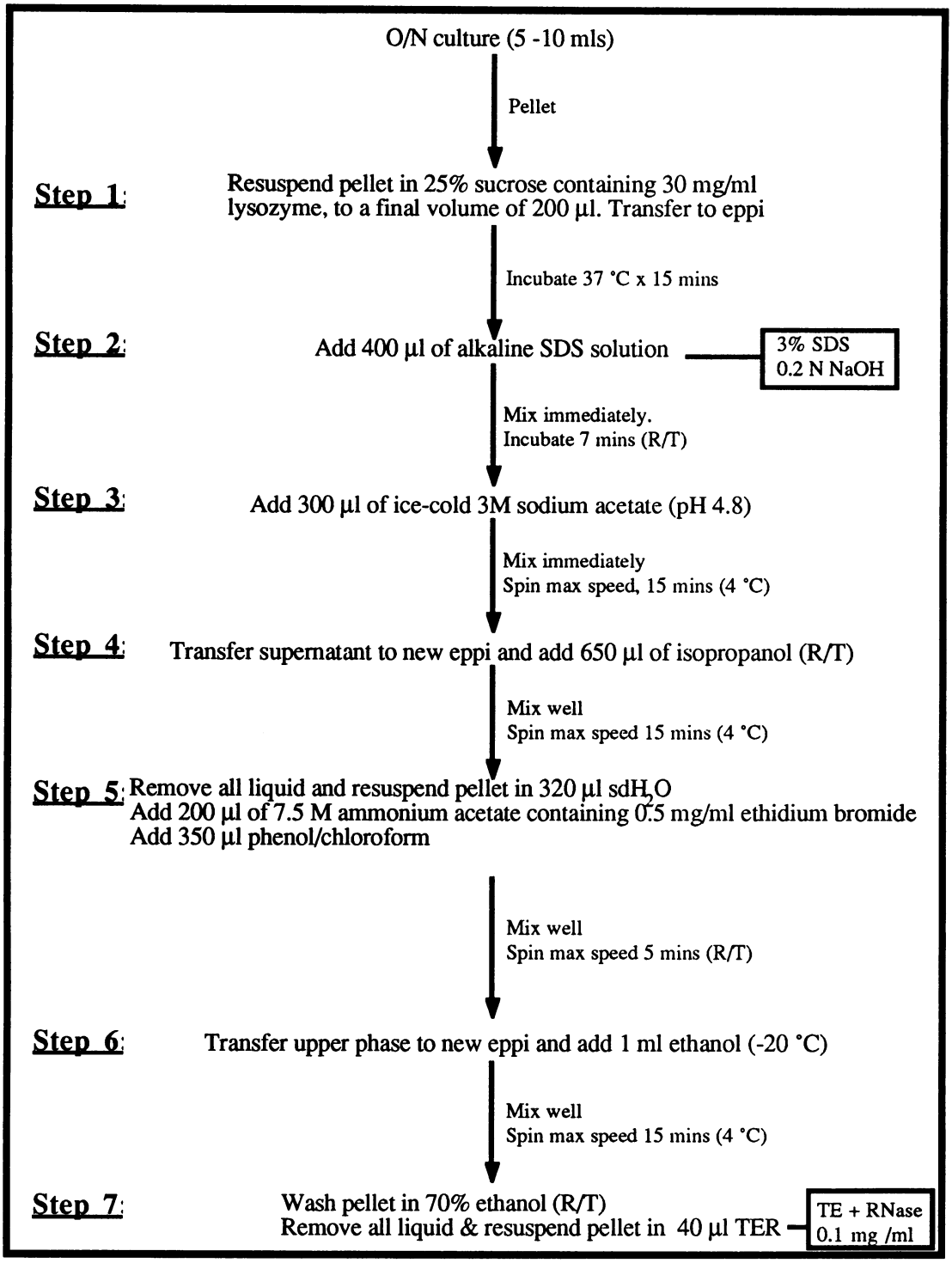


FIG. 1. Step-by-step outline of the plasmid mini-prep procedure developed in this study. See text for details. Eppi, Eppendorf tube; R/T, room temperature; sdH$_2$O, sterile distilled water; phenol-chloroform and TE (Tris-EDTA) buffer are as described in Sambrook et al. (13); O/N, overnight. Unless otherwise stated, solutions were made up in water.

plified the removal of the upper aqueous layer containing plasmid DNA. All the ethidium bromide was effectively separated from the DNA by this organic solvent extraction. The final plasmid DNA pellet was translucent in appearance, which is indicative of a clean plasmid preparation.

To determine whether a mini-prep resulted in sufficient plasmid DNA for restriction analysis, the high- and low-copy-number plasmid vectors (pIL252 and pIL253, respectively) were isolated from 10-ml cultures of *L. lactis* LM0230. In this host background, these plasmids were

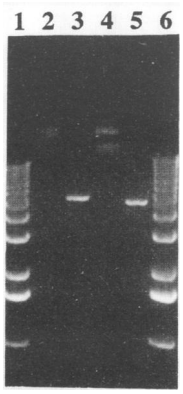


FIG. 2. Analysis of vector plasmids pIL253 and pIL252, isolated from *Lactococcus lactis* LM0230. Lanes 1 and 6, 1-kb ladder (GIBCO BRL); lane 2, pIL253, uncut; lane 3, pIL253 digested with *Eco*RI; lane 4, pIL252, uncut; lane 5, pIL252 digested with *Eco*RI.

estimated to be present at 6 to 9 and 45 to 85 copies, respectively (14). Figure 2 shows uncut and restricted pIL253 and pIL252 preparations. Both these vectors exist in *Lactococcus* spp. in multimeric forms, shown clearly in the uncut plasmid profiles (Fig. 2). The lanes represent either 4% of the pIL253 preparation or 20% of the pIL252 preparation, demonstrating that the procedure was very efficient at extracting plasmid DNA. Complete restriction of the plasmids was achieved in a low restriction digest volume (20 μl), demonstrating the purity of the preparations. Both of these plasmid preparations were also used directly in successful ligation and cloning experiments (data not shown).

While this procedure was developed initially to quickly analyze recombinant plasmids in lactococci, it was subsequently evaluated for examining plasmid profiles of different strains of *Lactococcus* and *Lactobacillus* spp. As shown in Fig. 3A and 4, representative plasmid profiles were obtained

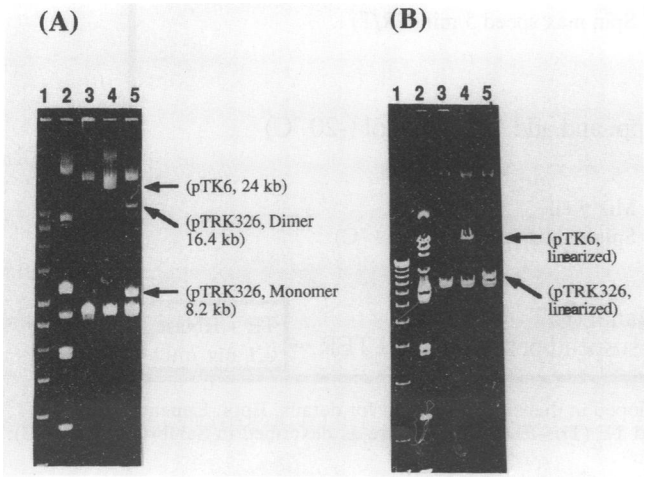


FIG. 3. (A) Plasmid content of lactococcal strains. Lane 1, supercoiled DNA ladder (GIBCO BRL); lane 2, NCK545; lane 3, NCK203; lane 4, NCK211 [NCK203 (pTK6)]; lane 5, NCK546 [NCK203 (pTRK326)]. The sizes of the recombinant plasmids pTK6 and pTRK326 are indicated. (B) Corresponding *Bam*HI restrictions of plasmid mini-preps. Lanes are the same as in panel A except for lane 1 (l-kb ladder; GIBCO BRL).

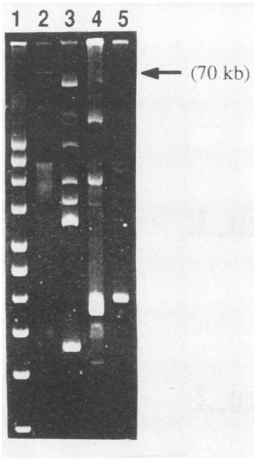


FIG. 4. Plasmid content of *Lactobacillus* spp. Lane 1, supercoiled DNA ladder (GIBCO BRL); lane 2, *L. acidophilus* C7; lane 3, *L. plantarum* NCK129; lane 4, *L. delbrueckii* subsp. *bulgaricus* 10A; lane 5, *L. johnsonii* NCK88. The position of the 70-kb plasmid in C7 is indicated.

from all strains tested. The recombinant plasmids pTK6 and pTRK326 were introduced previously into NCK203, and they were clearly visible in the uncut profiles shown in Fig. 3A. Restriction endonuclease digestion of both recombinant plasmids with *Bam*HI (a single site in both plasmids) resulted in complete digestion (Fig. 3B).

Relatively large plasmids can be isolated by this technique. A plasmid conservatively estimated to be 70 kb was readily isolated from *L. acidophillus* C7 and is shown in Fig. 4. The profiles of the *L. plantarum* and *L. bulgaricus* strains also compared favorably with the profiles reported previously for these strains (7). Total restriction of these plasmid preparations was also observed (data not shown).

These mini-preps were done with overnight stationary-phase cultures of *Lactobacillus* strains. Therefore, the protoplasting and lysis steps detailed in Fig. 1 were adequate when applied to lactobacilli and generated sufficient plasmid DNA for analysis. This mini-prep procedure should prove extremely useful for routine analysis of plasmids in lactic acid bacteria and possibly in other gram-positive bacteria.

This work was supported by project NC02168 of the North Carolina Agricultural Research Service.

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